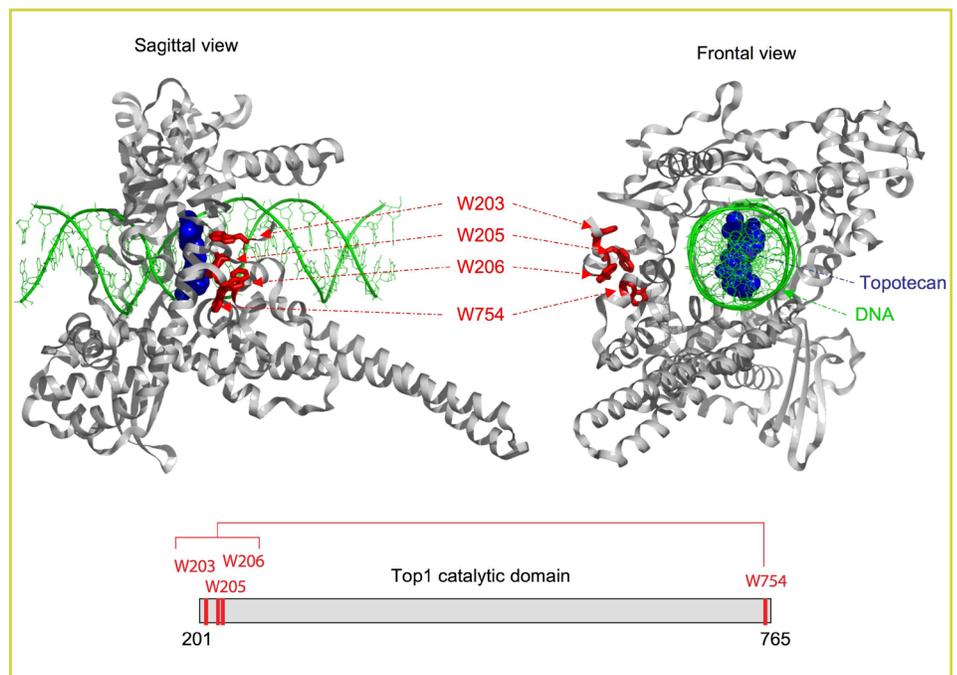


Trio of Tryptophans Aid in Camptothecin Therapy

On occasion, a normally desirable feature becomes not so desirable, making it necessary to find ways to work around it. The double helical nature of DNA helps it attain a compact, supercoiled state, which is essential for lengthy strands of DNA to reside in the nucleus as part of the chromosomes. During DNA replication and gene transcription, however, cellular machinery needs to access individual DNA strands; thus, the supercoiled, knotted state becomes undesirable. Just as a tangled telephone cord has to be manipulated, the DNA strands must be separated, unwound, and eventually joined together again.

The enzyme that helps supercoiled DNA relax during cell division and transcription is topoisomerase I (Top1). Top1 breaks one DNA strand and rejoins it after the DNA has relaxed. Top1 thus plays a vital role in cell division and transcription, and the structural integrity of the enzyme must be maintained to achieve this role. The enzyme is composed of two domains: the N-terminal domain (amino acids 1–200) and the functional domain (amino acids 201–765). The N-terminal domain helps transport the enzyme to the nucleus, where it performs its job, and the functional domain is largely responsible for binding to and relaxing DNA.



A three-tryptophan anchor on the functional domain of Top1 (red) is used by the enzyme to relax DNA. This anchor helps make the enzyme a good target for anticancer agents. The figure shows two views of how a Top1 inhibitor called topotecan interferes with Top1 as it tries to relax DNA.

As reported in the January 23, 2008, issue of *Biochemical Journal*, CCR researcher Yves Pommier, M.D., Ph.D., Chief of the Laboratory of Molecular Pharmacology, and his collaborator Gary Laco, Ph.D., of Lake Erie College of Osteopathic Medicine in Bradenton, Fla., studied the structure and function of Top1. In particular, they examined the junction region (amino acids 186–215) that bridges the N-terminal and functional domains. The researchers analyzed several

published structures and found that three tryptophans (a type of amino acid) at positions 203, 205, and 206 interact in a way that anchors one end of the functional domain to some of its other sub-domains, one of which contains the active site of the enzyme. When the three tryptophans were mutated, the resulting mutant Top1 had a different conformation and could not relax supercoiled DNA as efficiently. The mutant Top1 only removed from one to several supercoils

before releasing its hold on the DNA. In contrast, mutants missing all or part of the N-terminal domain—but retaining the three tryptophans—dissociated from the DNA only after the DNA was fully relaxed. These results illustrate the critical contributions of the tryptophans to the ability of the functional domain to carry out its job.

The relaxation of supercoiled DNA by Top1 is inhibited by the drug

camptothecin, an anticancer agent whose derivatives are currently used in cancer chemotherapy. The mutated Top1 is only partially inhibited by camptothecin, probably because the mutant enzyme dissociates from supercoiled DNA before it is fully relaxed, so there are fewer enzyme-DNA complexes for the drug to act on. The stabilizing effect of the three-tryptophan anchor on the functional domain of Top1 helps make the enzyme a good target for anticancer

agents. Knowledge about the structural features of Top1 that make it susceptible to inhibition by camptothecin and its derivatives may help researchers develop even more effective Top1 inhibitors.

Reference

Laco GS, Pommier Y. Role of tryptophan anchor in human topoisomerase I structure, function, and inhibition. *Biochem J* 2008 [Epub ahead of print].